Appendix 2.

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Presynaptic Regulation of Neurotransmitter Release

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Modulation of noradrenaline release from sympathetic nerve terminals by cholinomimetic drugs and cholinergic nerves

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Introduction

There have been several reviews dealing inter alia with the effects on sympathetic nerve terminals of acetylcholine and of other drugs affecting cholinergic mechanisms. Some of these effects were used to provide part of the arguments for advocating the cholinergic link hypothesis in noradrenergic transmission put forward by Burn & Rand, 1959, 1960b, 1962, 1965, see also Burn 1961, 1967, 1971, 1977a,b). It is not our present purpose to pursue this matter, and we refer those interested to various critical reviews (Ferry, 1966; Jaju, 1969; Campbell, 1970; Kosterlitz & Lees, 1972; Baldessarini, 1975; Westfall, 1977; Fozard, 1979; Muscholl, 1970). A further wave of interest in cholinoceptors on sympathetic nerve terminals was generated by the realization that neurotransmitter release could be modulated by activation of prejunctional or presynaptic receptors of various types (for reviews, see Rand, McCulloch & Story, 1975; Starke, 1977; Westfall, 1977; Vanhoutte, 1977; Vizi, 1979; Vanhoutte & Levy, 1980; Muscholl, 1980a; Vanhoutte, Vebeuren & Webb, 1982).

Cholinomimetic drugs are generally divided into those having muscarinic activity and those having nicotinic activity, but an important group has action on both types of cholinoceptors: in addition, certain cholinomimetic drugs have actions that do not fit comfortably into either category, and indeed some actions are unrelated to cholinoceptors. The main example of a drug acting on both types of cholinoceptors is acetylcholine; another is carbachol. Drugs acting predominantly or solely as muscarinic agonists are muscarine itself, methacholine and McN-A-343 (4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium). Drugs acting predominantly or solely as nicotinic agonists are nicotine

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itself, DMPP (N,N-dimethyl-N'-phenylpiperazinium) and phenocholine and certain of its analogues. The use of atropine to block muscarinic receptors and thereby reveal nicotinic effects is not without problems since in high concentrations atropine can block nicotinic receptors and also adrenoceptors; furthermore, antinicotinic drugs can block some of the actions of muscarinic agonists (see Rand & Stafford, 1967).

The effects of cholinomimetic drugs on sympathetic nerve terminals are complex. The main effects are a nicotinic stimulation of noradrenaline release, muscarinic and nicotinic inhibitions of noradrenaline release in response to depolarization of nerve terminal varicosities, and facilitation of stimulation-induced noradrenaline release by two mechanisms, one of which involves nicotinic receptors.

Nicotinic stimulation of sympathetic noradrenergic nerve terminals

Sympathomimetic effects of nicotine and of acetylcholine in the presence of atropine in isolated tissues have been noted by many workers and were at first ascribed to stimulation of sympathetic ganglion cells or chromaffin cells within the tissue (Dixon, 1924). Other early observations are listed in Table 1. It was later shown that such effects occurred in tissues entirely devoid of ganglion cells (Middleton, Oberti, Prager & Middleton, 1956; Thompson, 1958; Burn et al., 1959; Lee & Shideman, 1959; Daly & Scott, 1961; Bevan & Su, 1964; Lindmar, Löffelholz & Muscholl, 1968; Su & Bevan, 1970; Furchgott Steinsland & Wakade, 1975).

In most of the studies listed in Table 1, blockade of the sympathomimetic action by nicotinic antagonists was demonstrated. The sympathomimetic actions of nicotinic agonists were, in most cases, only slightly reduced by atropine in large doses, but then the responses to agonists of postjunctional adrenoceptors were also decreased. In some cases, the sympathometic effects of acetycholine, when used alone, were increased after atropine, as observed in the spleen of the cat (Brandon & Rand, 1961) and dog (Daly & Scott, 1961).

Loss of the sympathomimetic responses to nicotinic agonists has been reported after sympathectomy produced by pretreatment with 6-hydroxydopamine (Westfall, 1971b; Westfall & Brasted, 1972; Fozard & Mwaluko, 1976) or surgically (Burn et al., 1959; Lee & Shideman, 1959; Gillespie & MacKenna, 1960; Daly & Scott, 1961; Ferry., 1966); however, complete surgical denervation is difficult to achieve and did not always result in loss of sympathomimetic responses (Haney & Lindgren, 1945; Ginzel & Kottegoda, 1953; Burn et al., 1959).

Sympathomimetic responses to nicotinic agonists are reduced or abolished when the stores of noradrenaline in sympathetic nerve terminals are depleted by reserpine pretreatment (Burn & Rand, 1957, 1958a,b; Burn et al., 1959; Gillespie & MacKenna, 1960; Daly & Scott, 1961; Brandon & Rand, 1961; Lindmar, 1962; Bhagat, 1966; Su & Bevan, 1970; Westfall, 1971a,b; Westfall & Brasted, 1974; Steinsland & Furchgott, 1975a). In addition, drugs that block adrenoceptors block the sympathomimetic actions of nicotinic agonists, as has been established for β-adrenoceptor blockade in cardiac preparations (Lee & Shideman, 1959; Kukovetz, 1962;

Torchiana & Angelakos, 1963) and α-adrenoceptor blockade in vascular preparations (Burn & Dutta, 1948; Kottegoda, 1953b; Millsom, 1959; Steinsland & Furchgott, 1975a) and the spleen (Brandon & Rand, 1961; Daly & Scott, 1961).

Nicotinic agonists can also stimulate other types of nerve endings, including sensory nerve endings (see Fewings, Rand, Scroop & Whelan, 1966). The stimulation of non-noradrenergic, noncholinergic nerves in the guinea-pig taenia caeci by nicotine or DMPP produced a relaxation resembling the response to sympathetic nerve stimulation (Burnstock, Campbell & Rand, 1966).

Evidence that the sympathomimetic effects of nicotinic agonists is due to noradrenaline release

Release of the sympathetic transmitter during the cardiac stimulation produced by acetylcholine in the presence of atropine and other nicotinic agonists was demonstrated by Spadolini & Domino (1940), Hoffman, Hoffman, Middleton & Talesnik, (1945) and McNamara, Krop & McKay, (1948). The substance released was first identified as noradrenaline by Richardson & Woods (1959). The release of endogenous noradrenaline or of radioactivity after labelling sympathetic transmitter stores with (^{3}H)-noradrenaline by nicotinic agonists such as nicotine itself, acetylcholine (usually in the presence of atropine) and DMPP has since been demonstrated by many workers in cardiac preparations (usually perfused hearts), spleen, vascular preparations, intestinal preparations and vas deferens (see Table 2).

Comparison of the effects of nicotinic agonists and of electrical stimulation of sympathetic nerves.

Activation of nicotinic receptors on nerve terminals initiates action potentials (Ferry, 1963, 1966; Cabrera, Torrance & Viveros, 1966; Concha & Norris, 1968; Davey et al., 1968; Haeusler, Thoenen, Haefely & Huerlimann, 1968; Haeusler et al., 1969a,b; Krauss et al., 1970; Bevan & Haeusler, 1975). As with transmitter release produced by electrical stimulation of sympathetic nerves, that produced by nicotinic agonists is calcium-dependent (Lindmar et al., 1967; Haeusler et al., 1968; Westfall & Brasted, 1972; Fozard & Mwaluko, 1976).

Tetrodotoxin: Unlike the transmitter release produced by electrical stimulation of sympathetic nerves, the sympathomimetic actions of nicotinic agonists are generally reported to be not affected by blockade of propagation of action potentials by tetrodotoxin (Löffelholz, Lindman & Muscholl,1967; Haeusler et al., 1969b; Krauss et al., 1970; Westfall & Brasted, 1972; Fozard & Mwaluko, 1976; Jayasundar & Vohra, 1978; Lindamood, Johnson & Fleming, 1978; Su & Bevan, 1970; Toda, 1975; Furchgott et al., 1975). Therefore, noradrenaline release by nicotinic agonists could be said to occur in the absence of nerve impulses. However, Bell (1968) and Endoh, Tamura & Hashimoto (1970) reported that the effects of nicotine on various tissues were abolished by tetrodotoxin. The discrepancy may be explained by the finding of Furchgott et al. (1975) with the rabbit ear artery that tetrodotoxin blocked responses to low but not to high concentrations of nicotinic agonists, but Fozard & Mwaluko (1976) used a wide range of concentrations in rabbit heart without finding

such a differential effect. However, Sarantos-Laska, McCulloch, Rand & Story, (1981) showed that in rabbit atria which had been loaded with (^{3}H)-noradrenaline, tetrodotoxin blocked the release of radioactivity produced by electrical field stimulation and also that produced by 10 μM nicotine, which were similar being about 1% of the atrial content of (^{3}H)-noradrenaline but did not decrease the releases produced by 50 and 100 μM nicotine which were 9 and 16%, respectively, of the atrial content.

Adrenergic neurone blocking drugs. The sympathomimetic responses to nicotinic agonists, like responses to electrical stimulation of sympathetic nerves, are blocked by adrenergic neurone blocking drugs such as xylocholine, bretylium and guanethidine in cardiac preparations (Hukovic, 1960; Boyd, Chang & Rand, 1961), vascular preparations (Hukovic, 1960; Steinsland & Furchgott, 1975a), spleen (Boura & Green, 1959; Brandon & Rand, 1961), intestinal preparations (Burn & Gibbons, 1964) and vas deferens (Boyd et al. 1961). Adrenergic neurone blocking drugs have local anaesthetic properties, but the blockade of responses to nicotine in kitten atria and guinea-pig vas deferens was not mimicked by procaine (Boyd et al., 1961).

Metabolic profile of released noradrenaline. The noradrenaline released by electrical stimulation of sympathetic nerves appears in the fluid bathing the tissue, or in the effluent of perfused organs, largely as noradrenaline itself, whereas the release produced by tyramine-like displacing agents is mostly of metabolites (see Sarantos-Laska et al., 1980a). It was reported that nicotinic agonists released predominantly noradrenaline from the rat vas deferens (Jayasunder & Vohra, 1978) and rabbit pulmonary artery (Starke & Weitzell, 1978). The release produced by nicotine, DMPP and by acetylcholine in the presence of atropine from rabbit atria loaded with (^{3}H)-noradrenaline was compared with that produced by field stimulation by Sarantos-Laska et al. (1980a): for all four agents less than 10% of the release was as metabolites of (^{3}H)-noradrenaline. Since the nicotinic drugs did not have any inhibiting action on monoamine oxidase, the findings indicated that the release in all cases was by the same mechanism, presumably exocytosis. This is not in accord with the view that DMPP, but not nicotine, releases noradrenaline by a tyramine-like action from guinea-pig atria (Bhagat et al., 1967).

Autoinhibition of nicotinic stimulation. Another difference between the release of noradrenaline evoked by electrical stimulation and that evoked by nicotinic agonists is that the former is sustained at a steady level for long periods, whereas the latter decreases in the continued presence of the nicotinic agonist. The release of noradrenaline by nicotinic agonists has been described as explosive in nature since as much as 20% of the endogenous store can be released in 2 minutes (Löffelholz, 1970a). However, as noted above, in the continued presence of the agonist, the release wanes rapidly (Lindmar et al., 1967; Löffelholz, 1967; Su & Bevan, 1970; Nedergaard & Schrold, 1973). This phenomenon has been described as 'autoinhibition' (Löffelholz, 1970a) and has been attributed to desensitization of the nicotinic receptors (Steinsland & Furchgott, 1975b), probably resembling the better known effects on the nicotinic receptors of ganglion cells and motor end-plates. There is slow recovery of the responsiveness to nicotinic agonists after washout (Steinsland & Furchgott, 1975b).

Mechanisms of action of nicotinic agonists on sympathetic nerve terminals.

The full understanding of the effects of nicotinic agonists, as a class of drugs, on sympathetic nerve endings may require acceptance of the view that more than one mechanism may be involved, although one or other of the mechanisms may predominate depending on the particular agonist and the conditions under which its effects are observed. At least three possibilities must be entertained, namely: (1) a tetrodotoxin-sensitive stimulant action which results in the generation of action potentials and thence to transmitter release; (2) a tetrodotoxin-insensitive but Ca^{2+}-dependent effect which may involve a local non-propagated stimulation of excitation-secretion coupling; (3) a tyramine-like action involving neuronal uptake of the agonist and displacement of noradrenaline by a mechanism that does not involve Ca^{2+} entry.

Dual actions of acetylcholine on sympathetic nerve terminals

The nicotinic action of acetylcholine in releasing noradrenaline from sympathetic nerve terminals is complicated by the muscarinic action of acetylcholine in inhibiting the Ca^{2+}-dependent stimulation-induced release of noradrenaline. This was discovered by Muscholl and his group when they were studying the noradrenaline-releasing actions of acetylcholine and nicotine in the isolated perfused rabbit heart (see Muscholl, 1980b). They normally used atropine in their experiments, but on one occasion the atropine was omitted and they observed a reduction rather than an increase in noradrenaline release after administration of acetylcholine (Loffelholz et al., 1967).

When used alone, high doses of acetylcholine are required to elicit noradrenaline release, but in the presence of atropine, a greater release of noradrenaline is produced by lower concentrations of acetylcholine in perfused hearts of rabbit (Lindmar, et al. 1968; Löffelholz & Muscholl, 1969; Fozard & Muscholl, 1972) cat (Hauesler et al. 1968) guinea-pig (Westfall & Hunter, 1974) and chicken (Engel & Löffelholz, 1976).

The intracellular second messengers involved in the dual effects of acetylcholine in both inhibiting and stimulating the release of noradrenaline have been postulated by Weiner (1980), who suggested that the nicotinic stimulation could be due to the activation of adenylate cyclase and formation of cyclic AMP, whereas the muscarinic inhibition may involve the formation of cyclic GMP.

Muscarinic inhibition of sympathetic noradrenergic transmission

Inhibition of responses to sympathetic nerve stimulation by acetylcholine

The first observation on the blockade by acetylcholine of responses to sympathetic nerve stimulation was made by Brücke in 1935. He showed that the intradermal injection into the cat's tail of a small dose of acetylcholine produced local piloerection, but a large dose abolished the piloerection produced by sympathetic nerve stimulation, although the piloerector action of adrenaline was not affected. Similar findings were obtained with nicotine as well as

When the effect of nicotinic agonists on ganglion cells and motor end-plates has changed from stimulation to block, neurotransmission is blocked; however, this is not the case at sympathetic nerve endings since sympathetic nerve stimulation still produces noradrenaline release and effector responses when the autoinhibition of nicotinic excitation is complete (Löffelholz & Muscholl, 1969; Nedergaard & Bevan 1969; Löffelholz, 1970b; Su & Bevan, 1970; Ross, 1973; Steinsland & Furchgott, 1975b; Sarantos-Laska et al., 1980b). However, there are reports of blockade of responses to sympathetic nerve stimulation by nicotinic agonists (see below).

Modulation of nicotinic release by prejunctional α-adrenoceptors

The release of noradrenaline by propagated nerve impulses is reduced by activation of prejunctional α-adrenoceptors and is subject to feedback inhibition when the released noradrenaline activates prejunctional α-adrenoceptors (see Rand & Story, this volume). The release of noradrenaline by DMPP is reduced by the α-adrenoceptor agonist oxymetazoline, but feedback inhibition does not appear to be operating since the release is not increased by α-adrenoceptor blockade with phentolamine: in fact, it is reduced (Starke & Montel, 1974). The reduction in DMPP-induced noradrenaline release effect may possibly be due to the weak effect of phentolamine in blocking the neuronal uptake of DMPP.

Blockade of nicotinic stimulation of sympathetic nerve terminals

The sympathomimetic, noradrenaline-releasing effects of nicotinic agonists are not affected by atropine and other antimuscarinic drugs except in concentrations that exert effects other than blockade of muscarinic cholinoceptors (see Starke, 1977). However, in the case of a nicotinic agonist that also has muscarinic agonist activity, such as acetylcholine, atropine increases the effects by eliminating the counteracting inhibition of noradrenaline release (see below).

The effects of nicotinic agonists on sympathetic nerve terminals are generally blocked by drugs that block their effects on ganglion cells. However, there are exceptions; for example, in guinea-pig atria, the noradrenaline-releasing action of nicotine, but not that of DMPP, was blocked by hexamethonium (Bhagat et al., 1967). The explanation of this discrepancy appears to be that DMPP has a tyramine-like action which is not Ca^{2+}-dependent.

The sympathomimetic action and release of noradrenaline by nicotinic agonists is blocked by inhibitors of neuronal uptake, as shown with cocaine on responses to nicotine and DMPP in guinea-pig atria (Bhagat, 1966) and cocaine, desipramine and phenoxybenzamine in rabbit pulmonary artery (Su & Bevan, 1970). These observations led to the hypotheses (Su & Bevan, 1970; Bevan & Su, 1972) that the sympathomimetic effects of nicotinic agonists may be dependent on their uptake into the nerve terminals by the neuronal amine uptake process. However, later experiments failed to support this (Westfall & Brasted, 1972, 1974; Collett & Story, 1984).

acetylcholine by Coon & Rothman (1940) and these were confirmed by Burn et al. (1959) and Burn & Rand (1960a). Blockade by acetylcholine of responses to sympathetic nerve stimulation in various other preparations has been reported (Hukovic, 1959; Burn & Rand, 1960a; Comer & Di Palma, 1961; Hellmann, 1963). The effect was generally thought to be due to a nicotinic action of acetylcholine since nicotine exerted a similar action (see Burn & Rand,1965; Ferry, 1966), although Hukovic (1959) and Hellmann (1963) did note that the effect of acetylcholine was partly reversed by atropine. However, an analysis of the receptors involved that would be acceptable by modern standards has not been carried out.

Since there was blockade of responses to sympathetic nerve stimulation but responses to α-adrenoceptor agonists (adrenaline or noradrenaline) were relatively unaffected, it was deduced that the site of action was prejunctional, resulting in inhibition of stimulation-induced release of noradrenaline. Observations of this type can only be made in tissues in which the postjunctional effect of acetylcholine does not unduly complicate the issue, for example, either by producing the opposite effect to sympathetic nerve stimulation as in cardiac preparations, or by acting synergistically with adrenoceptor activators as in the vas deferens. Isolated vascular preparations afford the opportunity, and the striking inhibitory effect of acetylcholine on responses to sympathetic nerve stimulation was noted by de la Lande & Rand (1965), who observed that the effect was blocked by atropine, but they failed to identify it as prejunctional. Blockade of responses to sympathetic nerve stimulation without blockade of responses to α-adrenoceptor agonists are readily demonstrable in the rabbit isolated ear artery (Rand & Varma, 1970, 1971; Allen et al., 1972b, 1974, 1975; Hume de la Lande & Waterson, 1972; Steinsland, Furchgott & Kirpekar, 1973), in canine isolated blood vessels (McGiff, Burns & Blumenthal, 1967; Vanhoutte, Lorenz & Tyce, 1973), rat mesenteric arterial bed (Malik & Ling, 1969b; Leach & Zumani, 1969) and dog saphenous vein in situ (Vanhoutte & Shepherd, 1973). In these preparations, the inhibitory effect of acetylcholine is shared by other muscarinic agonists and is blocked by antagonists of muscarinic cholinoceptors. Therefore, the effect is clearly on muscarinic receptors and, as described below, inhibition of stimulation-induced release of noradrenaline has been demonstrated directly.

Inhibition of stimulation-induced noradrenaline release by acetylcholine and other muscarinic agonists.

Prejunctional muscarinic cholinoceptors subserving inhibition of release of noradrenaline from sympathetic nerve terminals were first clearly identified in rabbit isolated perfused hearts in which noradrenaline release was evoked by nicotinic agonists (Löffelholz et al., 1967; Lindmar et al., 1968). The observations led naturally to experiments in which noradrenaline release was evoked by sympathetic nerve stimulation, in which the inhibitory effects of muscarinic agonists were clearly demonstrated (Löffelholz & Muscholl, 1969; Löffelholz, 1970b; Fozard & Muscholl, 1972; Muscholl, 1973a,b; Fuder & Muscholl, 1974; Göthert, 1977). They have also been demonstrated in rabbit and guinea-pig isolated atria (Hope, McCulloch, Rand & Story, 1974; Story et al., 1975) and in isolated perfused hearts of the cat (Haeusler et al., 1968),

guinea-pig (Lindmar et al., 1968; Westfall & Hunter, 1974; Westfall & Brasted, 1974), chicken (Löffelholz, 1975; Engel & Löffelholz, 1976) and in the dog heart in situ (Levy & Blattberg, 1976; Lavallée, de Champlain, Nadeau & Yamaguchi, 1978).

Inhibition of nerve stimulation-induced noradrenaline release by acetylcholine and other muscarinic agonists has been demonstrated in various preparations of isolated blood vessels including the rabbit ear artery (Allen et al., 1972a; 1975; Steinsland et al., 1973; Rand et al., 1975; Story et al., 1975) and pulmonary artery (Starke, Endo & Taube, 1975c; Taube, Endo, Bangerter & Starke, 1976; Endo, Starke, Bangerter & Taube, 1977), and canine arteries and veins (Vanhoutte et al., 1973; Vanhoutte & Shepherd, 1973; Vanhoutte, 1974, 1977; Vanhoutte & Verbeuren, 1975, 1976a,b; Van Hee & Vanhoutte, 1978). Other preparations in which the effect has been demonstrated include the cat spleen (Kirpekar et al., 1972, 1975), guinea-pig vas deferens (Stjärne, 1975; Leighton & Westfall, 1976) and rabbit lung (Mathé, Tong & Tisher, 1977).

Mechanisms of muscarinic inhibition of noradrenaline release

Acetylcholine, and presumably other muscarinic agonists, inhibit the exocytotic release of noradrenaline since acetylcholine inhibits release not only of noradrenaline but also of dopamine-β-hydroxylase from guinea-pig heart (Langley & Gardier, 1974, 1977) and vas deferens (Leighton & Westfall, 1976). Noradenaline release that does not involve exocytosis is not inhibited by muscarinic agonists, as is the case with release produced by tyramine (Löffelholz & Muscholl, 1969; Vanhoutte et al., 1973; Vanhoutte, 1974) or by low Na^+ solutions (Dubey, Muscholl & Pfeiffer, 1975; Göthert, 1977; Muscholl, Ritzel & Rossler, 1979).

Exocyctotic release of noradrenaline is produced not only by electrical stimulation of sympathetic nerves but also by nicotinic agonists and by raising the K^+ concentration and thereby depolarizing the sympathetic nerve terminals. Noradrenaline release by nicotinic agonists is inhibited by muscarinic agonists as described above. It has also been shown that noradrenaline release evoked by raising the K^+ concentration is inhibited by muscarinic agonists in cardiac (Haeusler et al., 1968; Muscholl, 1973a; Dubey et al., 1975) and vascular (Vanhoutte & Verbeuren, 1976a; Verbeuren & Vanhoutte, 1976, Vanhoutte, 1977) preparations.

There is evidence that prejunctional muscarinic cholinoceptors modulate Ca^{2+} influx (Muscholl et al., 1979) since the inhibitory effect of muscarinic agonists on stimulation-induced noradrenaline release increased as the Ca^{2+} concentration was lowered (Dubey et al., 1975; Leighton & Westfall, 1976; Göthert, 1977; and was decreased by raising the Ca^{2+} concentration (Leighton & Westfall, 1976; Hope, McCulloch, Rand & Story, 1978). However, the Ca^{2+}-dependence of the effect was not observed in the dog saphenous vein (Vanhoutte, Verbeuren & Collis, 1977), in contrast to the above observations in rabbit cardiac and guinea-pig vas deferens preparations.

The rate of Ca^{2+} influx is proportional to the rate of stimulation of nerve terminals. This explains the findings that the degree of inhibition of stimulation-induced noradrenaline

release by acetylcholine and other muscarinic agonists is inversely proportional to the frequency of stimulation (Rand & Varma, 1970; Hume et al., 1972; Steinsland et al., 1973; Leighton & Westfall, 1976).

Nature of the prejunctional muscarinic cholinoceptor

The prejunctional and postjunctional cholinoceptors appear to be of the same type since the orders of activity of a range of agonists and antagonists in activating and blocking them, respectively, are the same (Muscholl, 1979; Fuder, Meiser, Wormstall & Muscholl, 1981, 1982a, 1985; Fuder, 1982; Lauer & Steinsland, 1983). However, in the rabbit ear artery, the antimuscarinic activities of gallamine, stercuronium and pancuronium in blocking the inhibitory effect of carbachol differed from the antimuscarinic activities found in other tissues (Li & Mitchelson, 1980; Leung & Mitchelson, 1983). Furthermore, the muscarinic agonist McN-A-343 was more potent on sympathetic prejunctional muscarinic receptors than on postjunctional muscarinic receptors in smooth muscle (Rand & Varma, 1971; Choo, Mitchelson & Vong, 1985). However, there may be a difference between the action of McN-A-343 and carbachol since pirenzepine selectively blocked the effect of McN-A-343 whereas pancuronium selectively blocked the effect of carbachol (Choo et al. 1985, 1986).

Location of muscarinic cholinoceptors on sympathetic nerve terminals

Destruction of the sympathetic terminal axons should result in loss of the prejunctional muscarinic cholinoceptors. This has been shown by measuring the binding of the receptor ligand (^{3}H)-quinnuclinidyl benzilate in preparations from the hearts of rats that have been pretreated with 6-hydroxydopamine compared with that in hearts from untreated rats (Story, Briley & Langer, 1979; Briley, Langer & Story, 1979; Sharma & Banerjee, 1977, 1978).

Effect of decentralization on prejunctional muscarinic cholinoceptors

Decentralization of the postganglionic noradrenergic neurones in the guinea-pig vas deferens resulted in loss of the prejunctional muscarinic cholinoceptors, and also of prejunctional α-adrenoceptors and prostaglandin receptors (see Westfall, 1977).

Interaction between prejunctional muscarinic cholinoceptors and other prejunctional receptors

α-Adrenoceptors. The inhibitory effect of acetylcholine on noradrenaline release from field stimulated rat atria was greater when short (16 pulses) than when long (60 pulses) trains of stimulation at 2 Hz were used (Loiacono, Rand & Story, 1985). This suggests that the effect is greater when there is less activation of prejunctional α-adrenoceptors. In fact, in the presence of phentolamine, the noradrenaline released by 60 pulses was considerably increased and was inhibited by acetylcholine to a much greater extent than in the absence of phentolamine. However, with 16 pulses of stimulation, phentolamine did not increase noradrenaline release nor did it alter the inhibitory action of

acetylcholine (Loiacono et. al., 1985). When prejunctional α-adrenoceptors were activated with an exogenous agonist (3,4-dihydroxyphenylimino-2-imidazolidine), the noradrenaline release by 16 pulses was decreased and acetylcholine failed to produce any further decrease (Loiacono et. al., 1985). In rabbit ear arteries, the release of noradrenaline evoked by sympathetic nerve stimulation was inhibited by acetylcholine, and this effect of acetylcholine was decreased by clonidine and increased in the presence of yohimbine or idazoxan, indicating that the inhibitory effect of activating muscarinic cholinoceptors was inversely proportional to the degree of activation of α-adrenoceptors (Loiacono et. al., 1985).

Angiotensin receptors. Blockade of muscarinic cholinoceptors enhanced the facilitatory effect of angiotensin II on stimulation-induced noradrenaline release in the rabbit isolated perfused heart (Garcia-Seville, Dubocovich & Langer, 1985).

Effects of nicotinic agonists resembling that produced by adrenergic neurone blocking drugs

The nicotinic agonist DMPP produces a slowly developing blockade of responses to sympathetic nerve stimulation without blocking postjunctional responses to adrenoceptor agonists in the guinea-pig vas deferens, rabbit ileum, rabbit ear artery and guinea-pig taenia caeci (Bentley, 1962; Wilson, 1962; Birmingham & Wilson, 1965; Burnstock, Campbell & Rand, 1966; Rand & Wilson, 1967) and blocks the stimulation-induced release of noradrenaline (Muscholl, 1970) by an action resembling those of guanethidine and bretylium. Actions resembling those of adrenergic neurone blocking drugs were described for nicotine itself (Burn & Rand, 1960a; Rand & Wilson, 1967) and phenocholine (Lederer, Rand & Wilson, 1970). The prototype drug from which the clinically useful adrenergic neurone blocking drugs such as bretylium and bethanidine were developed was xylocholine (choline 2,6-zylyl ether, TM10). It blocked responses to sympathetic nerve stimulation without blocking responses to adrenoceptor agonists (Burn & Rand, 1960a; Comer & Di Palma, 1961), and has a nicotinic stimulant activity. The conjunction of these two properties of xylocholine was used by Burn & Rand (1960b) as evidence for the cholinergic link hypothesis. One of the hallmarks of adrenergic neurone blocking drugs is that their blocking activity is reversed by amphetamine, and this has been demonstrated for DMPP, phenocholine and xylocholine by the workers mentioned above. In this connection, it is noteworthy that amphetamine also reverses the blockade of responses to sympathetic nerve stimulation produced by acetylcholine (Malik & Ling, 1969a) and McN-A-343 (Rand & Varma, 1971).

Facilitation of noradrenergic transmission release by cholinoceptor agonists

Facilitation by nicotinic agonists

Enhancement of responses to sympathetic nerve stimulation by nicotine and DMPP has been reported in various vascular preparations (Malik & Ling, 1969a; Nedegaard & Bevan, 1969a; Su & Bevan 1970; Steinsland et al., 1973; Steinsland & Furchgott, 1975a) and this is associated with, and presumably caused by, increased release of noradrenaline (Nedergaard & Schrold, 1977; Starke &

Weitzell, 1978; Kirpekar, Garcia & Prat, 1980). The effect of nicotine in enhancing the stimulation-induced release of noradrenaline, like the sympathomimetic action of nicotine, is transient (Sarantos-Laska et. al., 1980b). However, studies with cardiac preparations led to the conclusion that activation of nicotinic receptors does not modulate stimulation-induced noradrenaline release (Fuder, Siebenborn & Muscholl, 1982b; Westfall & Saunders, 1977).

The increase in stimulation-induced release of noradrenaline produced by nicotine is not due to blockade of uptake (Westfall & Brasted, 1972; Allen, Rand & Story, 1973b) and the enhancement of vasoconstrictor responses is not due to a postjunctional effect since nicotine did not affect responses to noradrenaline (Nedergaard & Bevan, 1969; Nedegaard & Schrold, 1977). However, DMPP is a potent inhibitor of neuronal uptake (Allen et al., 1973b), which might explain the enhancement of responses to sympathetic nerve stimulation that it produced in the rat perfused mesenteric artery preparation (Malik & Ling, 1969b).

Facilitation by muscarinic agonists

Enhancement of responses to sympathetic nerve stimulation by acetylcholine and other muscarinic agonists has been observed in various vascular preparations (Malik & Ling, 1969b; Rand & Varma, 1970; Allen et al., 1972a, 1975; Loiacono, Rand & Story, 1982). The concentrations of acetylcholine producing this enhancement are much lower than those having an inhibitory action. It is associated with an increase rather than a decrease in stimulation-induced noradrenaline release (Allen et al., 1972a, 1975) and is sensitive to changes in the extracellular Ca^{2+} concentration (Hope et al., 1978). The effect could not be identified as either muscarinic or nicotinic since it was not affected by either atropine or hexamethonium (Allen et al., 1975). The effect in the rabbit isolated ear artery observed by Allen et al. (1972a) could not be demonstrated by Hume et al. (1972) and it could not be demonstrated in other vascular preparations including rabbit pulmonary artery (Rand, et al. 1975; Taube et al., 1976; Endo et al., 1977) and canine blood vessels (Vanhoutte, 1977). A similar effect was observed in rabbit isolated atria (Hope et al., 1974; Story et al. (1975), but not in guinea-pig atria (Story et al., 1975) or whole rabbit heart (Muscholl, 1973b).

Rand & Varma (1970) noted two enhancing effects of acetylcholine on responses to sympathetic nerve stimulation in the rabbit ear artery as the concentration was increased: first, enhancement was seen with very low concentrations, then responses were decreased, and finally with very high concentrations, they increased again. The inhibitory effect but not the enhancement was blocked by atropine. Pilocarpine only produced slight increases in either the absence or presence of atropine.

The enhancement of responses to sympathetic nerve stimulation by cholinomimetic agonists can not be attributed generally to blockade of neuronal reuptake of noradrenaline since most cholinomimetic drugs are devoid of any appreciable effects on neuronal uptake as shown with acetylcholine with or without atropine (Lindmar, et al., 1968; Allen, et al. 1973b), nicotine (Nedergaard & Bevan, 1969; Westfall, 1971a; Allen et al., 1973b)

and methacholine and pilocarpine (Allen et al., 1973b). However, there are exceptions, for example, DMPP and McN-A-343 are nearly as potent as cocaine as uptake blockers (Allen et al., 1973b, 1974). This explains the effect of McN-A-343 in enhancing the efflux of noradrenaline and the positive inotropic response to sympathetic nerve stimulation in guinea-pig isolated atria (Allen et al., 1974) and rabbit perfused heart (Fozard & Muscholl, 1972, 1974).

The special case of McN-A-343

The muscarinic agonist McN-A-343 has actions requiring special consideration. It reduces vasoconstrictor responses to sympathetic nerve stimulation at low frequencies (< 10 Hz) in the rabbit ear artery (Rand & Varma, 1971) and this effect is blocked by atropine. However, in the presence of atropine, or with high frequencies of sympathetic nerve stimulation, McN-A-343 increases the vasoconstrictor responses (Rand & Varma, 1971). The inhibitory effect of McN-A-343 on responses to low frequency stimulation is due to a reduction in noradrenaline release (Allen et al., 1974). In guinea-pig atria, McN-A-343 has a positive inotropic action and increases the release of noradrenaline in response to sympathetic nerve stimulation (Bhagat, 1966; Allen et al., 1972a). These actions are not affected by either atropine or hexamethonium, but are abolished by reserpine pretreatment or β-adrenoceptor blockade. They are largely attributable to blockade of noradrenaline uptake, in which respect McN-A-343 has approximately the same potency as cocaine (Allen et al., 1973b). The uptake blocking action presumably also explains the increase in responses to sympathetic nerve stimulation seen in some cases in the rabbit ear artery. It may also explain the relaxation it produces in atropinized taenia caeci of the guinea-pig (Hobbiger, Mitchelson & Rand, 1969).

Although McN-A-343 acts on prejunctional muscarinic cholinoceptors in the rabbit ear artery to produce an atropine-sensitive reduction in responses to sympathetic nerve stimulation at low frequencies (Rand & Varma, 1970), it was reported to be without action on those receptors in rabbit heart (Fozard & Muscholl, 1972) or rabbit pulmonary artery (Nedergaard, 1980). The increase in stimulation-induced noradrenaline release produced by McN-A-343 in the presence of atropine in guinea-pig atria (Allen et al., 1972a, 1974) and rabbit perfused heart (Fozard & Muscholl, 1972) can be attributed to blockade of noradrenaline uptake (Bhagat, 1966; Allen et al., 1972a,b). However, in rabbit pulmonary artery it has an additional effect on responses to noradrenaline which was not due to either blockade of noradrenaline uptake or to stimulation of nicotinic receptors (Nedergaard, 1980).

Physiological significance of sympathetic prejunctional cholinoceptors on noradrenergic nerve terminals

If the activation of prejunctional cholinoceptors at sympathetic neuroeffector sites plays a physiological role, an endogenous agonist must be involved: the only feasible candidate is acetylcholine, and the most likely source is from cholinergic nerve terminals.

Cholinergic nerves running with the sympathetic nerve supply

Apart from the classical case of the cholinergic sympathetic innervation of the sweat glands (Dale & Feldberg, 1934), and the cholinergic supply to some vascular beds (see below), there is a considerable literature on the existence of a cholinergic component in the response to stimulation of the predominantly noradrenergic sympathetic innervation of various tissues (for reviews, see Burn & Rand, 1962, 1965; Campbell, 1970; Kosterlitz & Lees, 1972). Many of the reports are listed in Table 3.

Relationship between cholinergic and noradrenergic nerve terminals

Electron microscopy has revealed apposition of cholinergic and noradrenergic terminal varicosities suggestive of a functional relationship in vasa deferentia (Thoenen, Tranzer, Hurlimann & Haefely, 1966; Thoenen & Tranzer, 1968; Jones & Spriggs, 1975), irides (Richardson, 1964; Ehinger & Falck, 1966; Hökfelt, 1967; Ehinger, Falck, Persson, Rosengren & Sporrong, 1970a), nictitating membrane (Esterhuizen, Graham, Lever & Spriggs, 1968), some blood vessels (Graham, Lever & Spriggs, 1968; Iwayama, Furness & Burnstock, 1970; Nielsen, Owman & Sporrong, 1971; Edvinsson, Falck & Owman, 1977), and in the heart (Ehinger *et al.*, 1970; Cooper, 1965).

There is a close association of acetylcholinesterase with noradrenergic axons in many tissues (Eränko, Reckardt, Eränkö & Cunningham, 1970; Mottram, Ivens, Lever & Presley, 1973; Jones & Spriggs, 1975; Jacobowitz & Koelle, 1965; Waterson, Hume & de la Lande, 1970), but such an association is not ubiquitous (Jacobowitz & Koelle, 1965; Graham, Lever & Spriggs, 1968).

The possibility of noradrenergic-cholinergic cotransmission

Responses of the rabbit intestine to sympathetic nerve stimulation change during ontogeny from being predominantly cholinergic in newborn animals to noradrenergic within a few weeks (Day & Rand, 1961; Boatman, Shaffer, Dixon & Brody, 1965; Burn, 1968; Gulati & Panchal, 1978). This has been attributed to a slower development of cholinergic than of noradrenergic nerves (Pappano, 1977), but it may also be due to a change in the genetic expression of individual neurones during the process of maturation. The capacity to revert to a cholinergic from a noradrenergic mode of transmission was opened up by the observations of Holton & Rand (1962), who showed that the cholinergic component in the response of blood vessels in the rabbit ear to sympathetic nerve stimulation increased greatly after decentralization of the nerve supply. Studies with tissue cultures have shown that sympathetic postganglionic neurones that are destined to become noradrenergic exhibit plasticity during the early stages of their maturation and can be directed by choice of conditions to become cholinergic, noradrenergic or both (Patterson, 1978; Bunge, Johnson & Rees, 1978). It is possible, therefore, that some trace of a cholinergic mechanism may be present in mature noradrenergic neurones. Consideration of this possibility allows the speculation that muscarinic cholinoceptors on noradrenergic nerve terminals are a vestigial remnant of autoreceptors from the stage during development when the nerves were cholinergic.